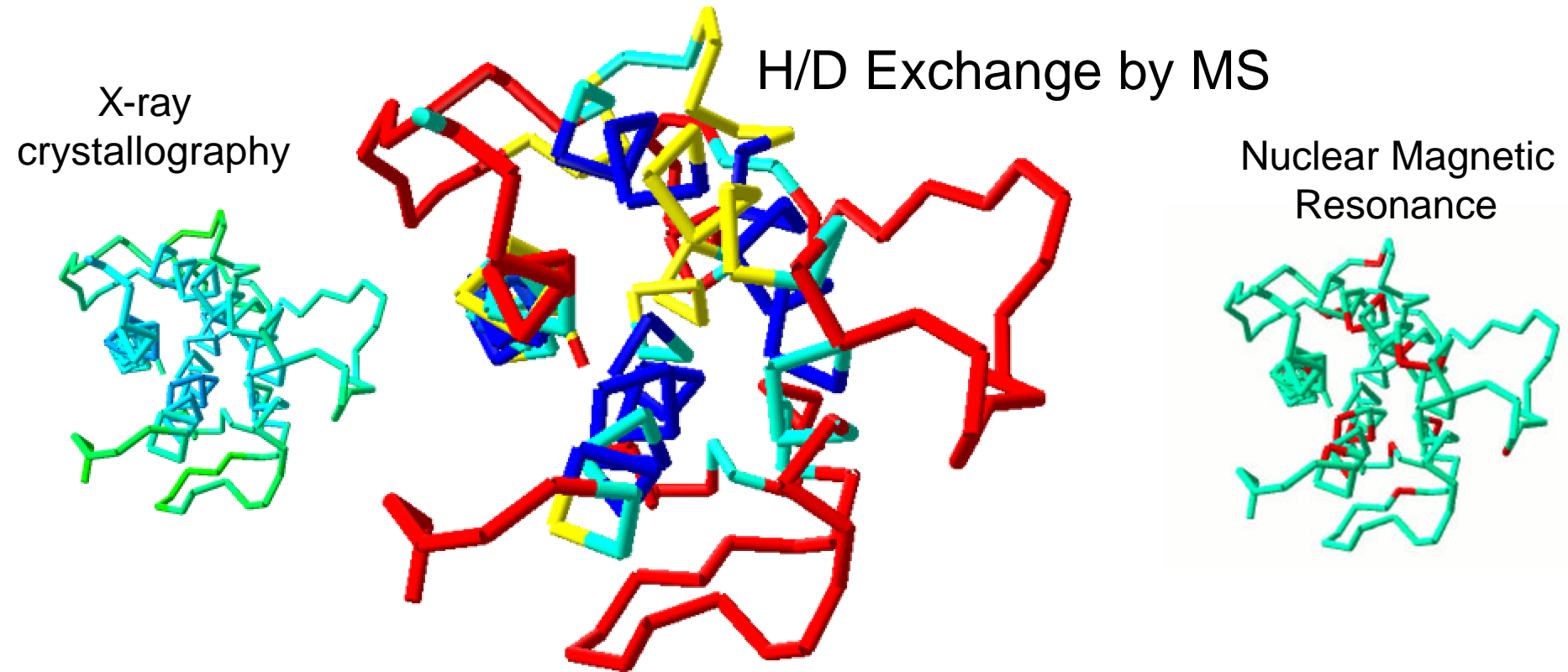


Hydrogen/Deuterium Exchange to Quantitate Protein Structure Similarity

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Methods for High Resolution Analysis of Protein Structures

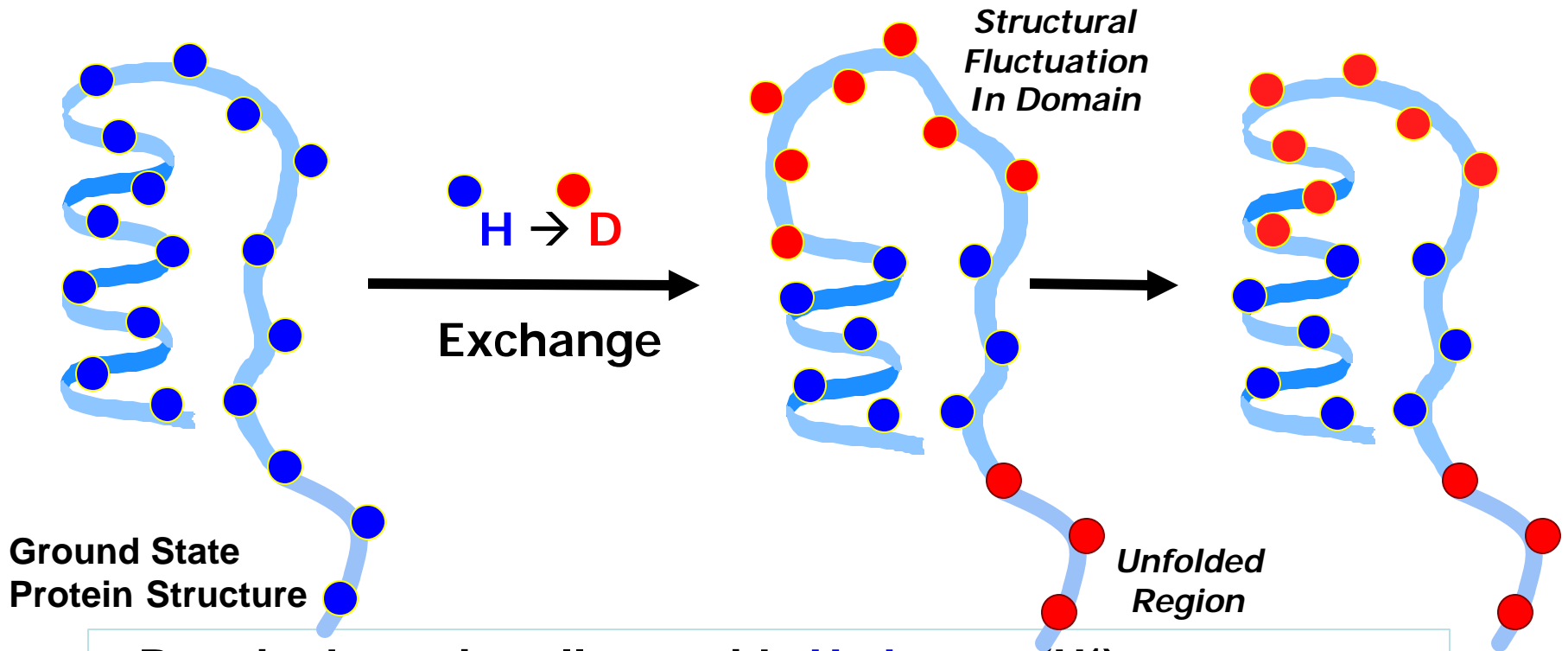


Only the Mass Spectrometry method can be widely applied to solution samples of unlabeled proteins.

Questions addressed today

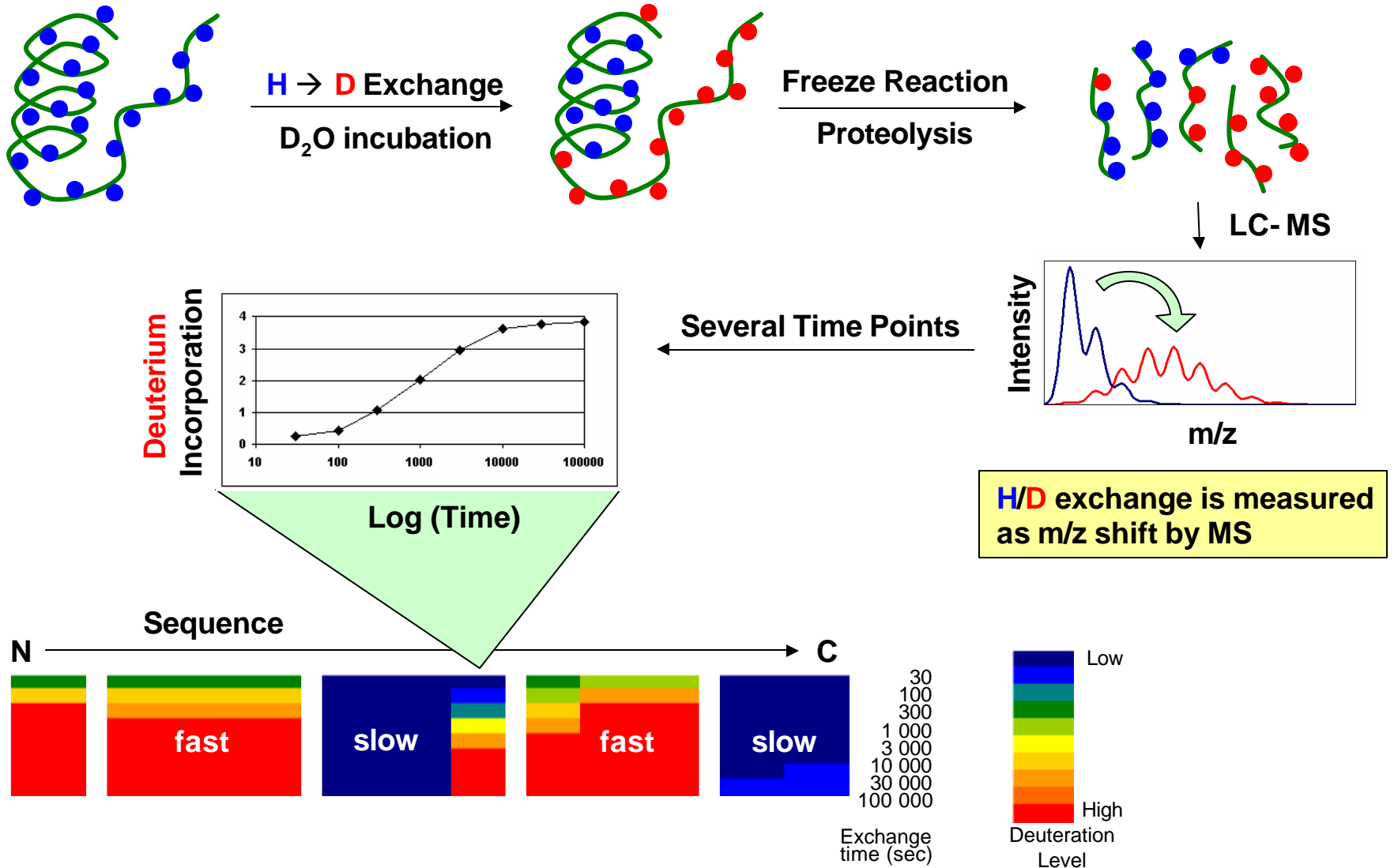
- Is the H/D exchange method suited to the assessment of structural comparability of biopharmaceuticals, i.e. does this method provide reliable information on protein composition and conformation?
- What structural information is provided by the H/D exchange method?

H/D Exchange Rates Provide a Sensitive Fingerprint of Protein Solution Structure



- Protein dynamics allow amide **Hydrogen** (H^1) atoms to exchange for **Deuterium** (H^2) atoms.
- Exchange rates vary over 10 000 000 fold in domains, and 50-100 fold in unfolded regions.

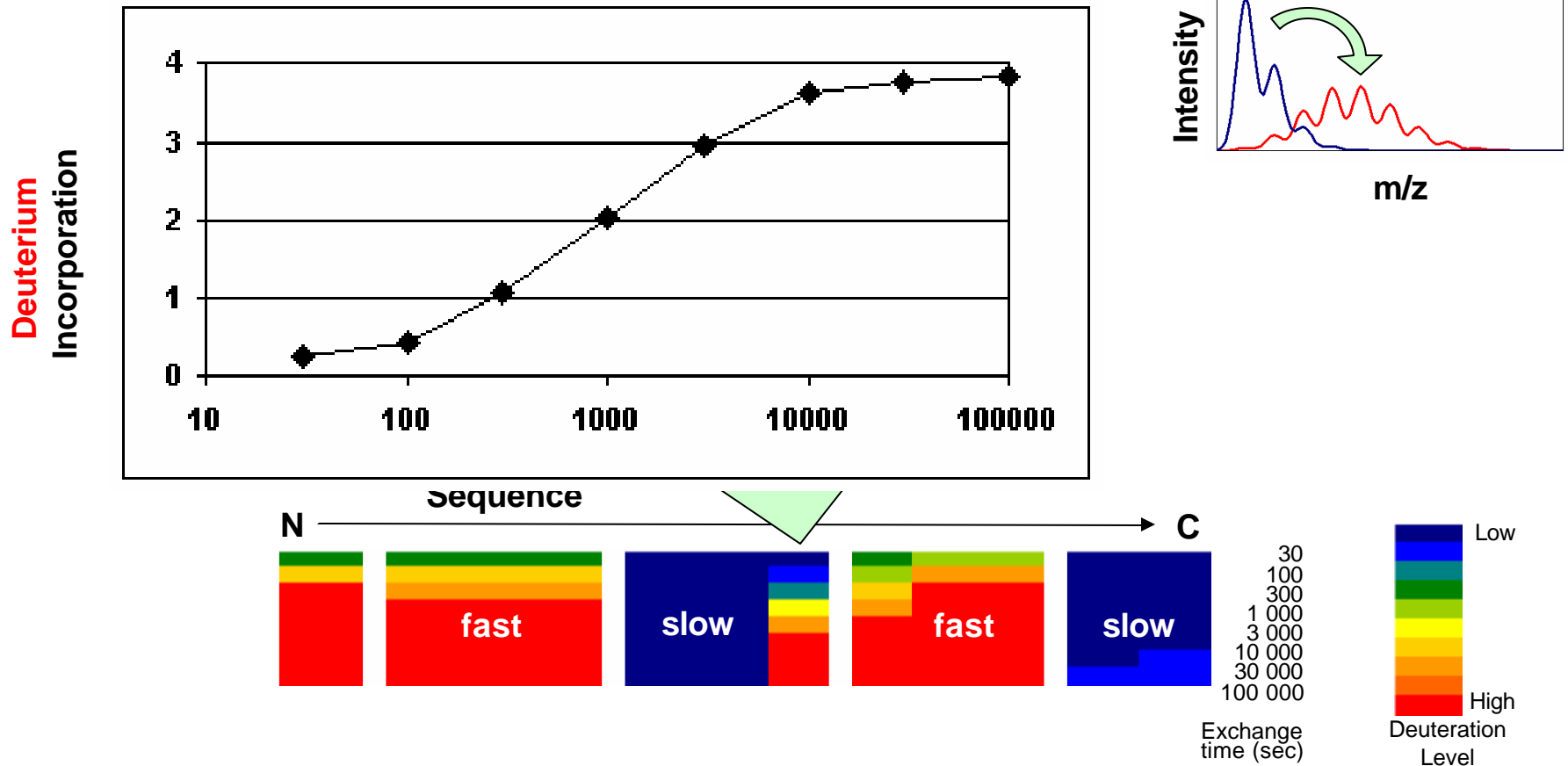
Measure of H/D Exchange at High Resolution



Comparison of Deuterium Buildup Curves

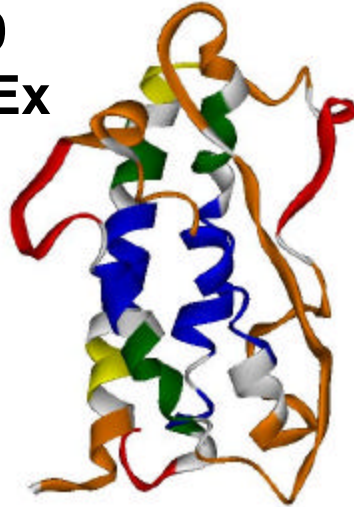
allows accurate assessment of protein conformation in

- 1) protein:ligand complexes
- 2) genetically modified or engineered proteins
- 3) proteins in different solvent systems.

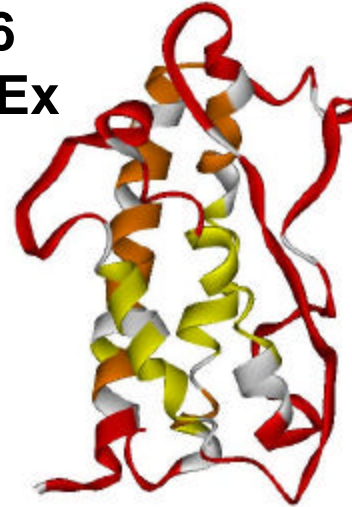


H/D exchange determinations by MS and NMR are comparable

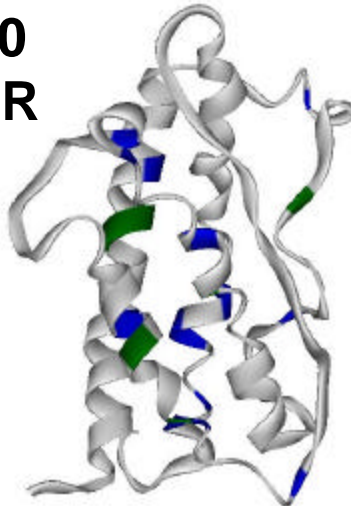
pH 7.0
by H/D-Ex



pH 2.6
by H/D-Ex



pH 7.0
by NMR



pH 2.6
by NMR

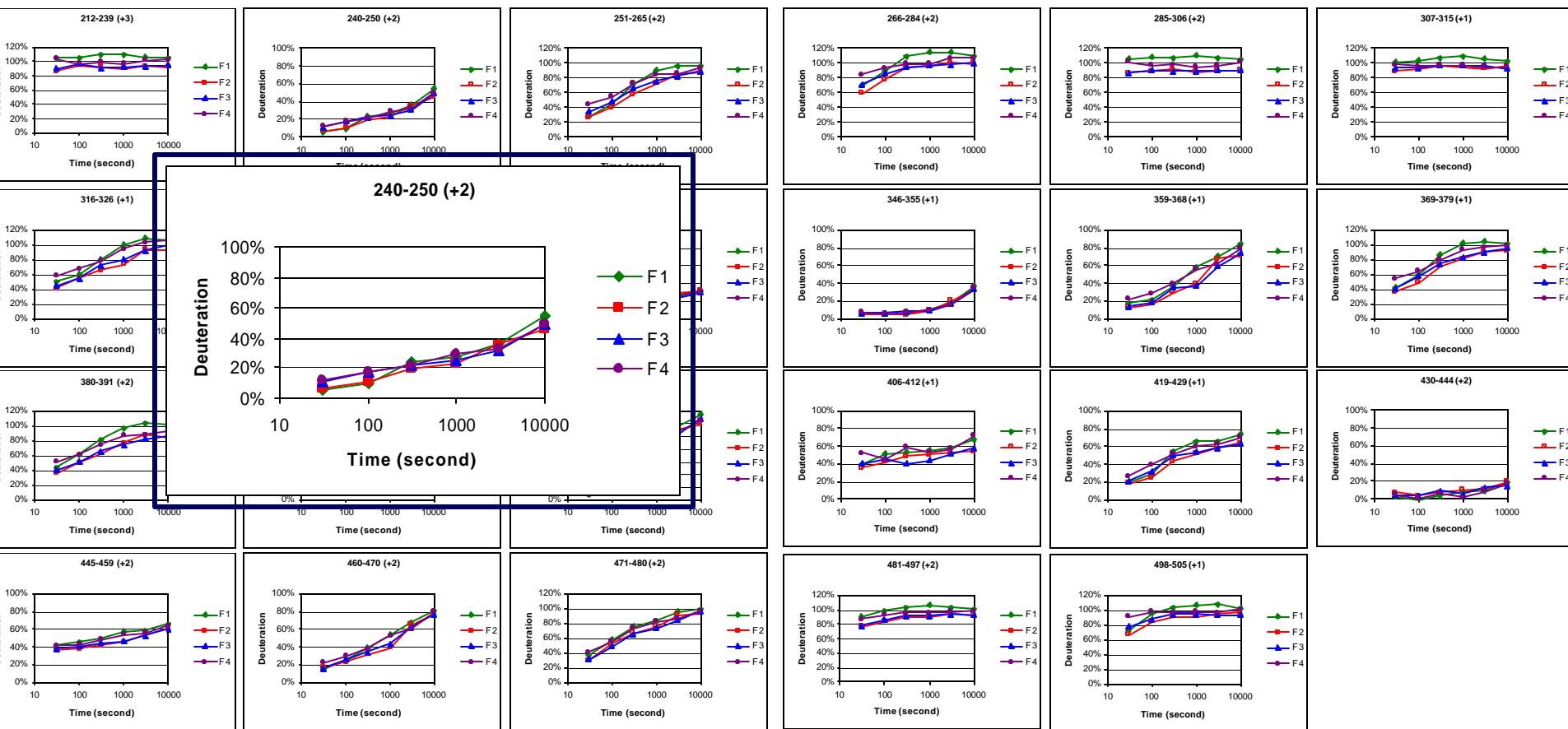


Some Applications of H/D Exchange

- **Protein engineering** - to assess changes in native protein fold when modifications are made to the amino acid sequence.
- **Ligand Mapping** – to localize sites of ligand interaction as an aid to structure-based design
- **Ligand Classification** – to categorize ligands by exchange pattern and, for example to distinguish between full and partial agonists of nuclear hormone receptors

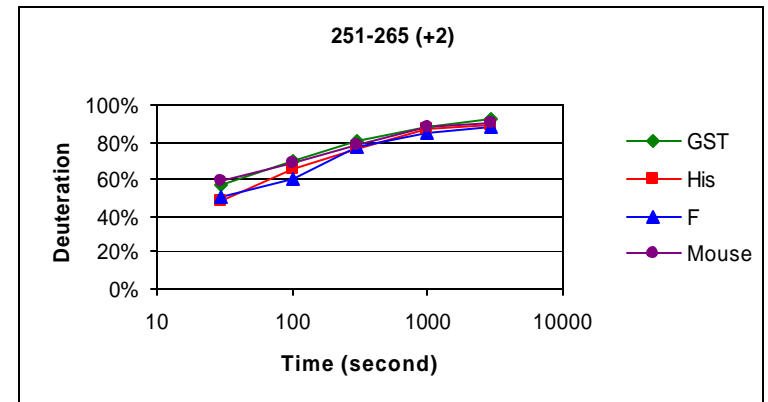
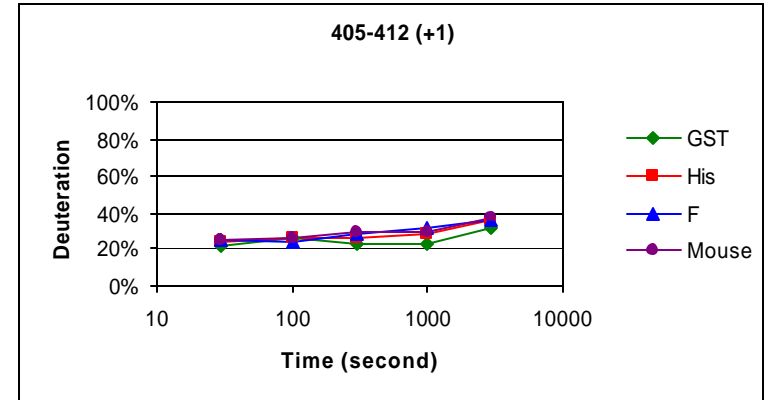
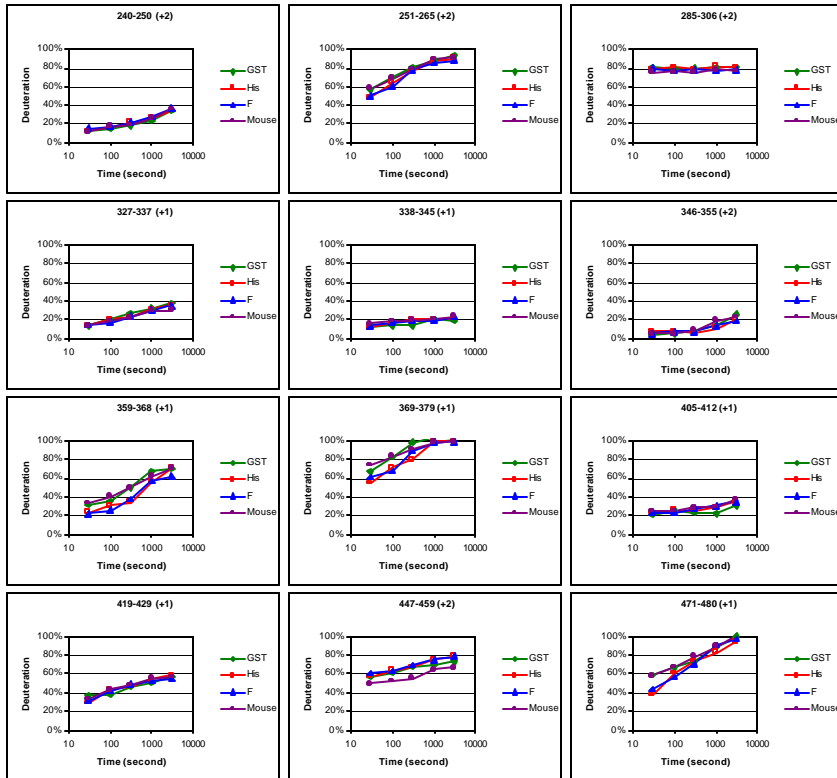
Reproducibility of H/D Exchange Experiments

Reproducible analyses for several runs over a month.



Comparison of Protein Samples

Similar exchange patterns for four variants indicate that a similar fold is retained in all.



Summary

- What information is provided by the H/D exchange method?

H/D exchange rates for defined segments throughout the protein, that provide a very sensitive measure of protein structure in solution.

- Is the method suited to the assessment of structural comparability of biopharmaceuticals?

Yes